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14. ABSTRACT:

Transition to a neuroendocrine prostate cancer (NEPC) phenotype has emerged as an important mechanism of treatment resistance to androgen receptor (AR) therapies for patients with metastatic prostate cancer. During the course of this Award, I have performed extensive, first –in-field molecular characterization of metastatic tumor biopsies from patients with castration resistant adenocarcinoma and neuroendocrine prostate cancer (Beltran et al, *Nature Medicine, 2016*). Whole exome, transcriptome, and CpG DNA methylation integrative analyses point to key drivers of NEPC including loss of RB1 and TP53, gain of MYCN, overexpression of BRN2, and epigenetic changes. Clonality analysis of serial tumor biopsies in individual patients provides new insights into mechanisms of progression, favoring a model most consistent with divergent clonal evolution of NEPC from an adenocarcinoma precursor. Through preclinical analyses, we have better characterized mechanisms of transdifferentiation (Dardenne*, Beltran* et al, *Cancer Cell* 2016; Bishop et al, *Cancer Discovery* 2016; Mu et al, *Science, in press*). Also as part of this Award, I have evaluated circulating tumor cells (CTCs) from patients treated with abiraterone and enzalutamide for emergence of NEPC CTC characteristics and found that up to 10% harbor NEPC-like CTCs (characterized by low AR, smaller morphology, loss of CK), and the presence of NEPC CTCs was associated with poor prognostic features (Beltran et al, *Clinical Cancer Research, 2016*). Circulating tumor DNA analysis is ongoing. I have also started to look even earlier in prostate cancer progression evaluating high risk prostate cancers and patients treated with neoadjvuant therapy on the CALGB90203 Phase 3 trial for emergence of NEPC features and harbingers of early resistance. These studies have potential clinical implications for early detection, prognostication, and identification of patients less likely to respond to subsequent AR-targeted therapies.

15. SUBJECT TERMS: Prostate Cancer, AR independence, Neuroendocrine prostate cancer, Treatment resistance, Circulating tumor cells, Biomarkers

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1. **INTRODUCTION:** Although patients with castration resistant prostate cancer (CRPC) have, by definition, castrate levels of circulating testosterone, most advanced prostate tumors continue to remain dependent on signaling from the androgen receptor (AR). Hence, most CRPC tumors are not truly hormone refractory. Based on this understanding, several new highly potent AR-targeted therapies have entered widespread clinical use for the treatment of patients with metastatic CRPC. While exciting, these drugs are not curative, and all patients ultimately develop resistance. In most cases, AR continues to remain active. However a subgroup of patients treated with AR therapies develop rapid progression and clinical features suggestive AR independence including low PSA progression and visceral metastases. Metastatic biopsies in this subgroup have revealed an emergence of tumor morphologic characteristics consistent with small cell carcinoma/neuroendocrine prostate cancer (NEPC). The goal of this Award is to systematically evaluate mechanisms of NEPC progression using deep sequencing techniques of metastatic tumor biopsies and non-invasively using liquid biopsies including circulating tumor cells (CTCs).

2. **KEYWORDS:** advanced prostate cancer, androgen receptor, resistance, abiraterone, neuroendocrine prostate cancer, circulating tumor cells, genomics, biomarkers

3. PROJECT SUMMARY:

Aim 1. To identify molecular determinants of acquired resistance to potent AR targeted therapies. The working hypothesis of this Aim is that advanced prostate tumors acquire genetic alterations in response to newer potent AR targeted therapies that enable them to continue to grow and proliferate. We will perform massively parallel whole exome sequencing of tumor tissue from abiraterone resistant prostate cancers to determine the spectrum of mutations associated with resistance to AR targeted therapies.

Aim 2. To prospectively evaluate circulating tumor cells (CTCs) from patients receiving potent hormonal therapies for acquisition of gene alterations in response to therapy. The working hypothesis of this Aim is that evaluation of CTCs may provide a non-invasive method to detect genomic alterations of key genes that occur before or may be acquired on therapy that predict response or resistance. We will analyze CTCs from patients prior to starting abiraterone, during treatment, and at progression for gene amplification of AR, Aurora kinase A, and N-myc, and correlate with clinical response to therapy.

Aim 3. To evaluate high-risk primary prostate tumors for mutations that may predispose to resistance to AR targeted therapy with comparison to matched metastatic tumors. The working hypothesis of this Aim is that specific genetic alterations occur early and predispose to the development of treatment resistance to AR targeted therapies, and these may be detected at the time of initial diagnosis in high-risk primary prostate tumors.

Aim 1 Progress: I have been systematically evaluating patients at different time points during treatment with potent AR targeted therapy and during progression from a hormone naive prostate adenocarcinoma to an AR-driven castration resistant adenocarcinoma (CRPC-Adeno) and/or AR –independent castration resistant neuroendocrine prostate cancer (CRPC-NE). Metastatic biopsies have not been considered standard of care for patients with advanced disease; therefore this effort has required prospective enrollment of patients on a research protocol with informed consent. I developed an IRB protocol at WCMC to perform metastatic biopsies and whole exome sequencing (WES) and other molecular analyses of tumor and germline DNA from patients with advanced disease and to follow patients prospectively to evaluate for response to subsequent therapies, optional re-biopsy at progression, long term follow up for PFS and OS endpoints, and optional participation in a rapid autopsy program. The design and initial results of this protocol were reported in Beltran et al, *JAMA Oncology* 2015. Somatic and germline results of enrolled CRPC patients pre and post abiraterone or enzalutamide were also included as part of the International SU2C-PCF Prostate Dream Team and recently published in Robinson et al, *Cell* 2015 and Pritchard et al, *NEJM* 2016. In addition, as study chair of a multi-center Phase 2 trial of the aurora kinase A inhibitor MLN8237 for patients with neuroendocrine prostate cancer,

I have obtained 60 pre-treatment metastatic tissue biopsies and blood samples from NEPC patients and reported initial clinical trial results and correlative studies during this grant period as an oral abstract presentation at ESMO Annual Meeting, Copenhangen, 2016 (Beltran at al, ESMO 2016; manuscript is in preparation).

During this year's grants period, we reported an integrative analysis of 114 metastatic tumor specimens from 81 patients including 51 patients with clinical and histologic features of castration resistant adenocarcinoma (CRPC-Adeno), 30 with castration resistant neuroendocrine prostate cancer (CRPC-NE) as confirmed by pathologic consensus criteria (Epstein et al, AJCP 2014), and 17 patients with multiple tumor biopsies (Beltran et al, *Nature Medicine 2016*). Biopsies were obtained from a wide range of metastatic sites, with a predominance of bone biopsies in CRPC-Adeno compared to CRPC-NE **(Figure 1a).** As expected, CRPC-NE demonstrated on average lower protein expression of the AR by immunohistochemistry**.** We also quantified AR signaling status by mRNA and observed overall lower AR signaling in CRPC-NE compared to CRPC-Adeno **(Figure 1b)**; however, there was significant overlap with a wide range of values observed within each subtype, suggesting that AR signaling alone is insufficient for subtyping metastatic tumors**.**

The mutational landscape of CRPC-NE was similar to CRPC-Adeno **(Figure 1c),** but also consistent with published studies of CRPC-NE including enrichment of *RB1* loss *(*deleted in 70% of CRPC-NE and 32% of CRPC-Adeno, p=0.003) and mutation or deletion of *TP53* (66.7% CRPC-NE versus 31.4% CRPC-Adeno, p=0.0043). Loss of *RB1* is common in primary small cell prostate and lung carcinomas, and promotes small cell carcinoma pathogenesis when concurrent with *TP53* mutation; in our series, concurrent *RB1* and *TP53* loss was present in 53.3% of CRPC-NE vs.13.7% of CRPC-Adeno (p<0.0004).

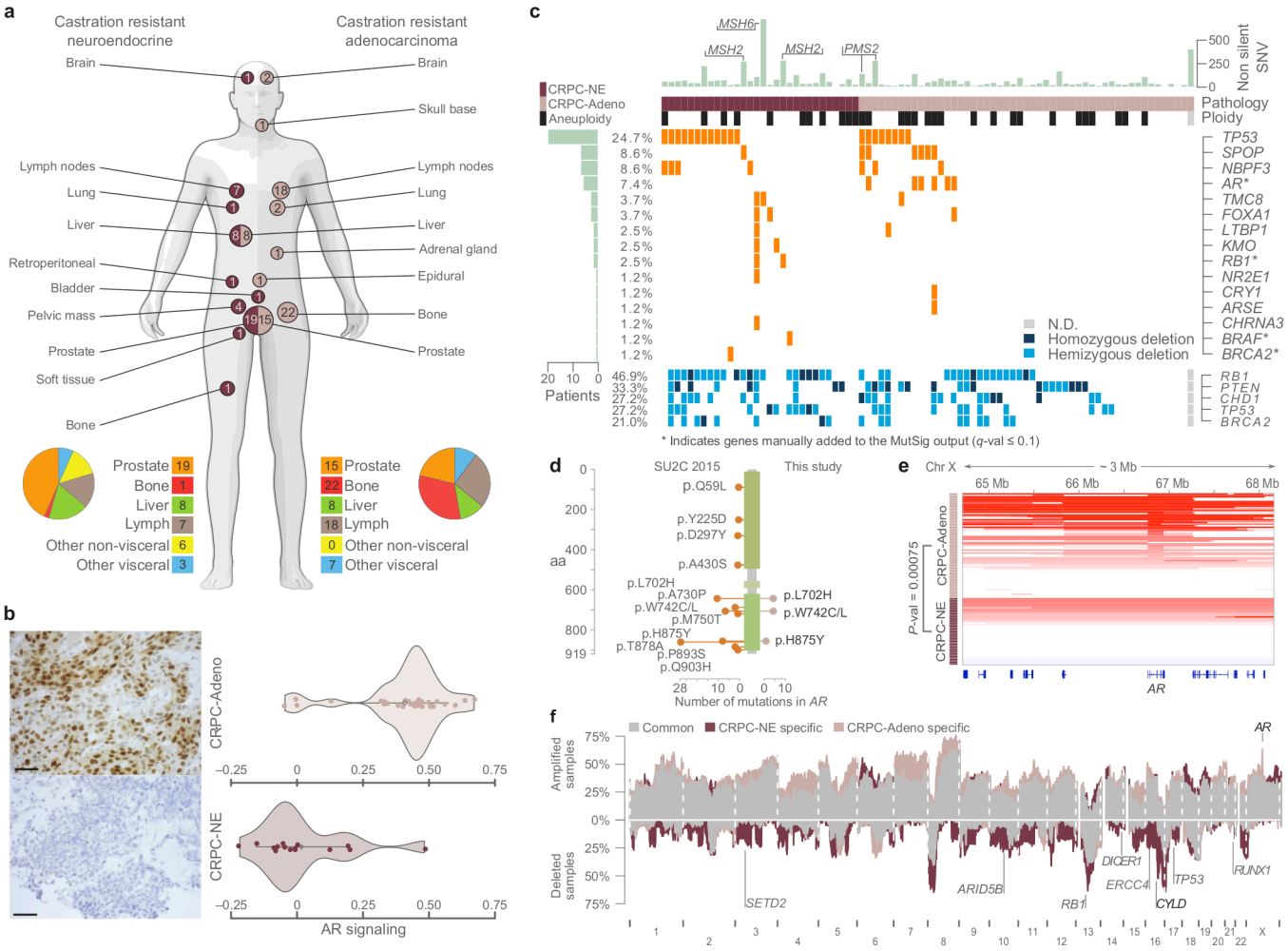


Figure 1. Clinical and mutational profile of the cohort. (**a**) Schematic illustrating sites of biopsy for CRPC-NE (dark pink) and CRPC-Adeno (light pink) subgroups. Numbers in circles indicate numerosity of samples from each site. (**b**) AR signaling (right) based on abundance of mRNA transcripts included in the AR signaling signature described in ref 19. Violin plots show the density of AR signaling. Each dot represents a sample; diamonds and solid lines represent the mean and 95% confidence interval, respectively. Representative immunohistochemistry (left) shows AR protein expression. Scale bars, 50 μm. (**c**) Significantly mutated genes. Each row represents a gene and each column an individual subject. Top light green bars correspond to the total number of non-silent SNVs in an individual. Left light green bars indicate the number of subjects harboring non silent corresponding mutations in the genes indicated on the right. Bottom panel reports the copy number status of selected genes. (**d**) Genomic location of AR mutations in samples from SU2C-2015 and this study. (**e**) Copy number status of AR locus. Color intensity and location are indicative of level and focality of amplification. (**f**) Frequency of copy number aberrations; concordant fractions (gray), CRPC-NE specific (dark pink) and CRPC-Adeno specific (light pink). Data adjusted for tumor ploidy and purity. Highlighted genes are significantly preferentially aberrant in one class and demonstrate concordant differential mRNA levels (for DNA and mRNA: FDR <= 10% for deletions and p-value <= 1% for amplifications).

In 2016 and in collaboration with Charles Sawyer lab (MSKCC), we have helped further elucidate the role of RB1 and TP53 in patient cohorts and their contribution towards driving AR independent resistance to AR therapies in CRPC (Mu et al, *Science, in press*).

Another distinguishing feature of CRPC-NE compared to CRPC-Adeno was a paucity of somatic alterations involving the AR gene ($p<0.0001$). Genomic amplification, activating point mutations, and splice variants involving the AR are commonly observed in CRPC-Adeno and associated with treatment resistance to AR-directed therapies (Robinson et al, Cell 2014). This observation was confirmed in our cohort; 29 cases showed *AR* focal amplification or point mutation and 21 cases had alterations in known AR co-activators (*FOXA1, NCOR1/2, ZBTB16*). In contrast, AR point mutations were notably absent in CRPC-NE and gains when present were of low level and explained by tumor polyploidy **(Figure 1d).** Although potentially affected by differences in prior therapies, we speculate that the absence of AR genomic alterations in CRPC-NE may be due to clonal selection of non-amplified CRPC-Adeno tumor subpopulations through selective pressure (in the context of AR-directed therapies). As a follow-up to this observation, in 2016 I am working on circulating tumor DNA evaluation of this same cohort and extended to more patients (n=100) and in preliminary analysis observed enrichment of AR alterations in CRPC-Adeno vs. CRPC –NE (Aim 2).

While informative, the observed DNA changes did not appear to fully explain the clinical aggressiveness of CRPC-NE. We therefore posited that this phenotype may also be mediated by epigenetic changes. Towards this end, we generated data to evaluate CpG-rich methylation genome wide by single cytosine resolution DNA methylation (eRRBS). In contrast to the largely similar genomic data, the CRPC-NE and CRPC-Adeno subtypes showed strong epigenetic segregation by unsupervised analysis using unselected methylation sites **(Figure 2a).** This raised the possibility that the transition to, or advent of, the CRPC-NE subtype is associated with epigenetic dysregulation. In fact, the epigenetic signal comprised an even stronger classifier than standard pathologic classification, as evidenced by the fact that it encompassed three cases that were initially binned as adenocarcinoma based on standard pathology**.** All three of these patients demonstrated radiographic progression in the setting of a stable or low serum level of the androgen-regulated protein prostate specific antigen (PSA). These data suggest that clustering prediction based on DNA methylation may provide additional information associated with AR independence and CRPC-NE that improves on tumor morphology. In 2016, I have been extending upon these observations to assess the functional role of DNA methylation changes in driving AR cistrome changes and downstream CRPC-NE features and evaluating intra-patient tumoral DNA methylation heterogeneity across metastatic sites at time of rapid autopsy (ongoing work).

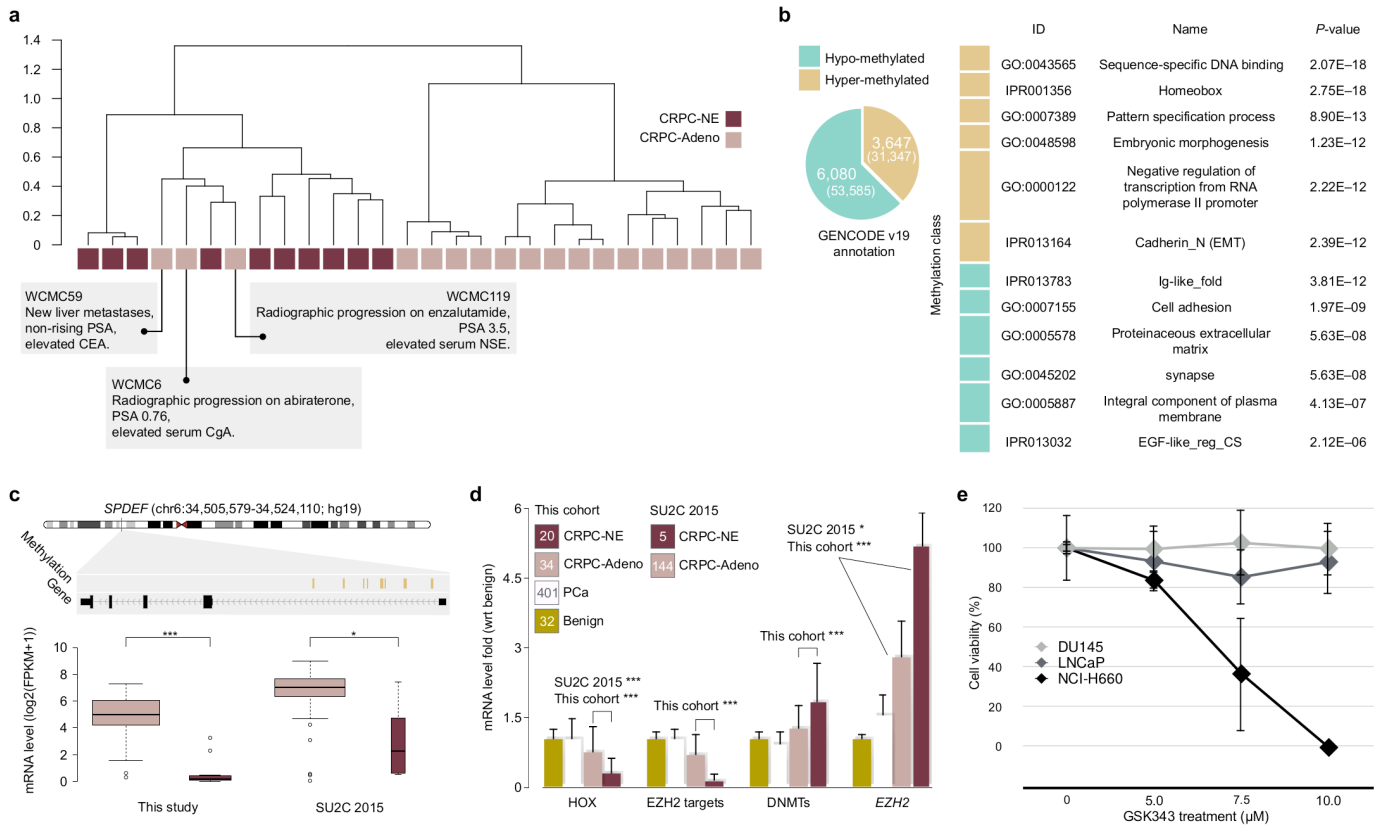


Figure 2. Methylation analysis of CRPC-NE and CRPC-Adeno. (**a**) Hierarchical clustering of 28 eRRBS samples data using (1 - Pearson's correlation) as distance measure on unselected sites. Clinical features of outlier cases are described. (**b**) Left, pie chart showing the number of differentially methylated genes, identified by annotating hyper- and hypo- methylated loci (number is reported between parentheses) on GENCODE version 19. Right, table shows a selection of terms enriched by differentially methylated genes. (**c**) Top, genome track of *SPDEF*. Hyper-methylated loci are reported in the annotation track. Bottom, box plot of expression levels of *SPDEF* samples for This Study (left) and SU2C/PCF 2015 (right) cohorts. (**d**) Bar plots highlight the effect of *EZH2* transcription activity across 487 samples with different pathology classification. The bars are relative to the mRNA level fold (with respect to benign prostate tissue samples) of homeobox genes under-expressed in CRPC-NE versus CRPC-Adeno (FDR < 0.1); a selection of *EZH2* target genes (*DKK1*, *NKD1*, *AMD1*, *HOXA13*, *HOXA11*, *NKX3-1*); DNA methyltransferase genes - indicated as DNMTs (*DNMT1*, *DNMT3B*, *DNMT3A*, *DNMT3L*); *EZH2*. Significance of differences between CRPC-NE and CRPC-Adeno subgroups are shown (max $P = 3*10^{-5}$ for DNMTs). When significant, p-values in SU2C/PCF cohort are shown. The number of samples for each pathology classification is reported inside the square symbols of the legend. (**e**) Cell viability in prostate adenocarcinoma cell lines (DU145, LNCaP) the neuroendocrine prostate cell line NCI-H660 assessed at 48 hours after treatment with escalating does of the EZH2 inhibitor GSK343 (5, 7.5, 10uM).

The DNA methyltransferase EZH2 was significantly overexpressed in CRPC-NE compared to CRPC-Adeno ($p<10^{-6}$, Wilcoxon test) **(Figure 2d)** and verified at the protein level. Furthermore, EZH2 target genes are also downregulated in CRPC-NE. Treatment of cell lines with the EZH2 inhibitor GSK126 resulted in a preferential decrease in cellular viability in NCI-H660 compared to other prostate cancer cell lines with significant down-regulation of CRPC-NE associated genes after treatment including CD56, MYCN, and PEG10.
In 2016, I have since been exploring the role of EZH2 as a potential target for patients with CRPC-NE by using preclinical models in my lab (Puca et al, AACR 2016) and in the clinic as Co-Investigator of the Phase 1 trial of GSK126 for solid tumors (we enrolled 3 CRPC/NEPC patients in 2016) with pre-treatment metastatic biopsies.

Based on the current gap in the clinical and molecular assessment of CRPC-NE, we developed a 70 gene molecular classifier to potentially improve upon the often challenging clinical diagnosis of CRPC-NE that relies on pathologic features. This integrated neuroendocrine prostate cancer (NEPC) classifier was developed by exploiting expression data of genes prioritized by genomic, transcriptomic or epigenomic status and demonstrated both a precision and recall of >0.99 in identifying CRPC-NE in our discovery cohort. Interrogation of transcriptome data of 683 prostate samples using datasets from The Cancer Genome Atlas (TCGA *2015*), Grasso et al 2012 (Michigan 2012), Robinson et al 2014 (SU2C/PCF, 2015), and internal

published data (Beltran et al 2011) revealed an elevated CRPC-NE score in up to 8% of metastatic tumors (n=191) and none of treatment naïve prostate adenocarcinoma (n=460) or benign prostate (n=32). Of those with markedly elevated CRPC-NE score, we reviewed the pathology and found over 80% had pathologic features of CRPC-NE. These findings warrant larger prospective clinical evaluation to verify whether this classifier could be useful as a potential prognostic or predictive biomarker (associated with lack of response to AR therapies). Incorporation of different layers helps apply the classifier to different datasets when only parts are available (DNA, RNA, or methylation) and paves the way for future studies that might apply the classifier to other methods (such as circulating tumor DNA, *see results of Aim 2*). For instance, if CRPC-NE alterations could be detected earlier during CRPC-Adeno disease progression, such individuals could be selected for CRPC-NE-directed rather than AR-targeted systemic therapies or co-targeting therapeutic approaches.

Since reporting these discoveries and our recent publication of this initial work in February 2016 (Beltran et al, Nature Medicine 2016), I have continued to delve deeper into the mechanisms underlying NEPC transdifferentiation and have worked in my laboratory and through collaborative efforts to identify the neuronal transcription factor BRN2 as an important driver (Bishop et al, *Cancer Discovery* 2016), elucidate the combined role of p53 and Rb1 (Mu et al, *Science, in press*), and the role of N-myc in promoting NEPC in cooperation with EZH2 (Dardenne, Beltran (co-first author) et al, *Cancer Cell,* 2016). I have also used metastatic biopsies from patients enrolled as part of this Aim to develop patient derived organoid models of NEPC using protocols we initially developed in collaboration with MSKCC (Gao et al, Cell 2014). These organoids recapitulate the metastatic tumor biopsy genomics (Puca et al, manuscript in preparation) and retain therapeutic drug response as the patients (Beltran et al, ESMO 2016). Overall, this Aim has fueled my lab's basic and translational research in NEPC and has facilitated a number of academic collaborations and high impact publications in 2016.

BRN2 in NEPC. In collaboration with Amina Zoubeidi's lab at Vancouver Prostate Cancer who developed multiple enzalutamide –resistant preclinical models, one of which was associated with NEPC features including suppressed AR signaling and upregulation of NEPC markers (called 42D), BRN2 was identified as the most highly upregulated genes in 42D (Fig 3). BRN2 is a neuronal transcription factor and master regulator. Through a series of experiments combined with clinical samples BRN2 was identified as a key regulator of NEPC transdifferentiation (Fig 4). This work was recently published (Bishop et al, *Cancer Discovery* 2016).

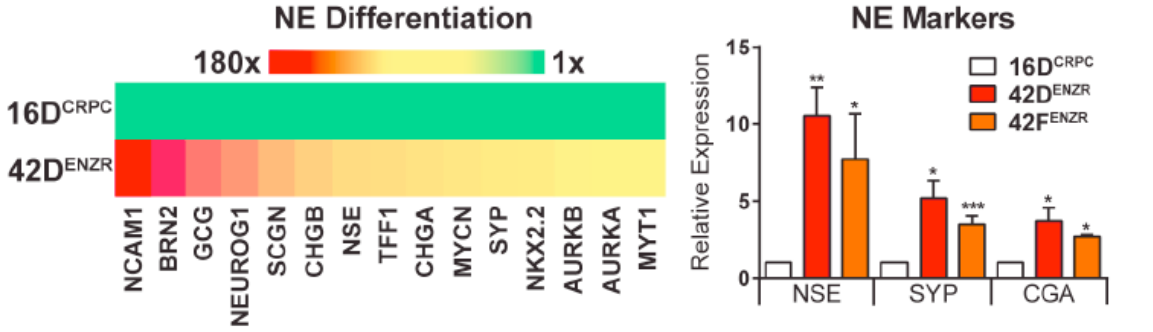
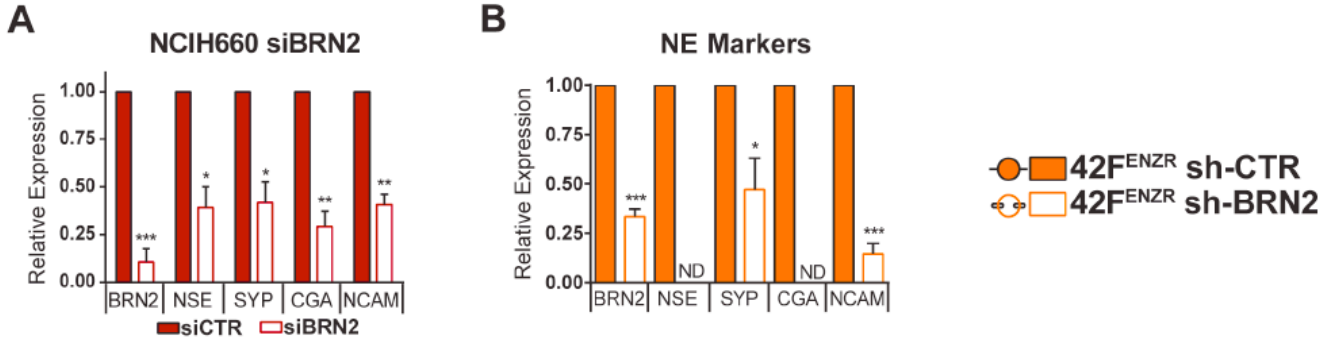


Figure 3: AR non-driven ENZR cells display a NE differentiation signature and increased levels of the neural transcription factor BRN2. (A) Heat map showing fold increase in reads per million of genes involved in NE differentiation in 42DENZR cells compared to 16DCRPC (=1). **(B-C)** Relative mRNA expression of **(B)** NSE, SYP, CGA and **(C)** NCAM1 in 42DENZR and 42FENZR cells compared to 16DCRPC



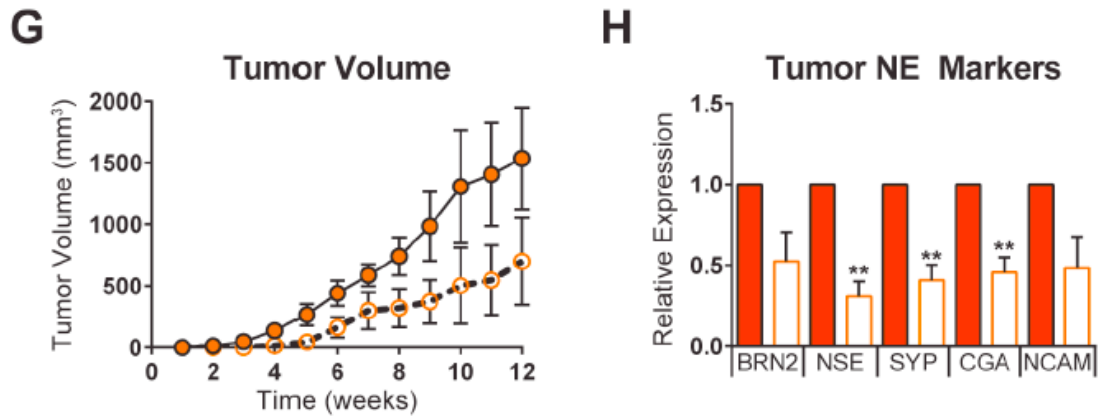
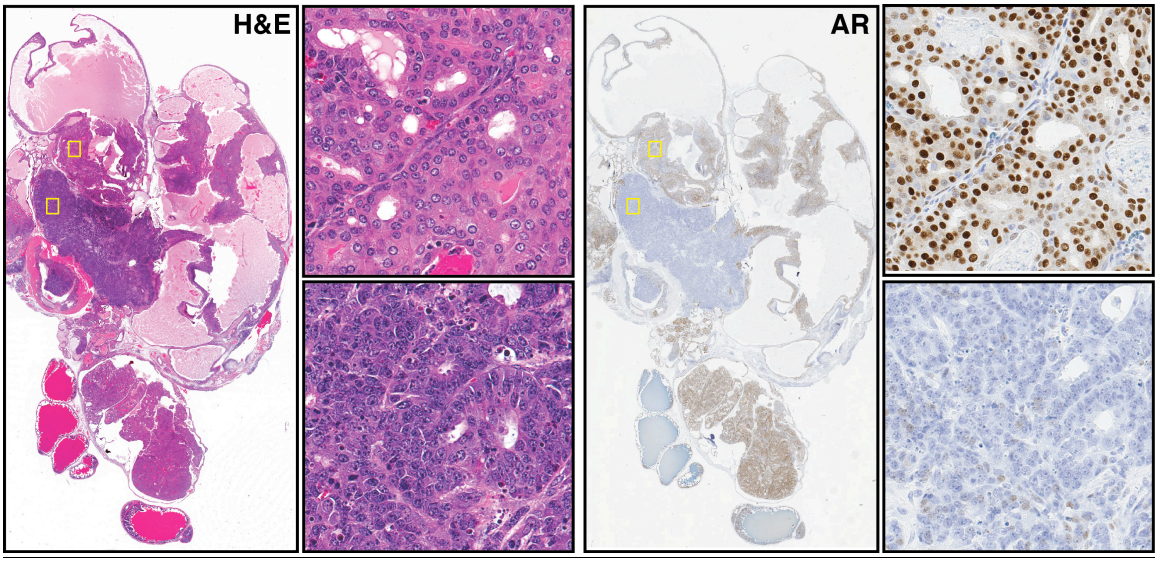


Fig 4. Relative mRNA expression of BRN2 and NE markers in **(A)** NCIH660 cells transfected with BRN2 siRNA (siBRN2) compared to control (siCTR=1) and **(B)** 42FENZR cells with stable BRN2 knockdown (sh-BRN2) compared to control transfected cells (sh-CTR) (=1). **(C)**Relative proliferation, 72 hours after seeding in NCIH660 cells transfected with BRN2 siRNA (siBRN2) compared to control (siCTR=1). **(D-F)** Relative proliferation **(D)**, Relative wound density in one dimensional scratch assay **(E)** and number of cells migrated through matrigel boyden chamber **(F)** in 42FENZR cells with stable BRN2 knockdown (sh-BRN2) compared to control transfected cells (sh-CTR) **(G)** Tumor volume of 42FENZR sh-CTR and 42FENZR sh-BRN2 xenografts grown in vivo (n=10). **(H)** Relative mRNA expression of BRN2 and NE markers in 42FENZR sh-BRN2 vs. sh-CTR xenografts (=1) harvested at 12 weeks post-inoculation. Graph represents pooled data from 6 sh-BRN2 and 6 sh-CTR tumors.

N-myc drives NEPC In collaboration with D Rickman at Weill Cornell, we developed a GEMM model *Pb-Cre+/-; Ptenf/f; LSL-MYCN+/+* that developed NEPC at 3 months (Figure 5, below). Photomicrograph images of H&E stained or AR IHC prostate tissue below show invasive, AR-positive adenocarcinoma foci and AR-negative NEPC foci. This work was recently published (Dardenne*, Beltran* (co-first author) et al, *Cancer Cell* 2016).



Using this GEMM model and multiple N-myc models we found that N-Myc interacts with EZH2 to drive transcriptional program (Fig 6)

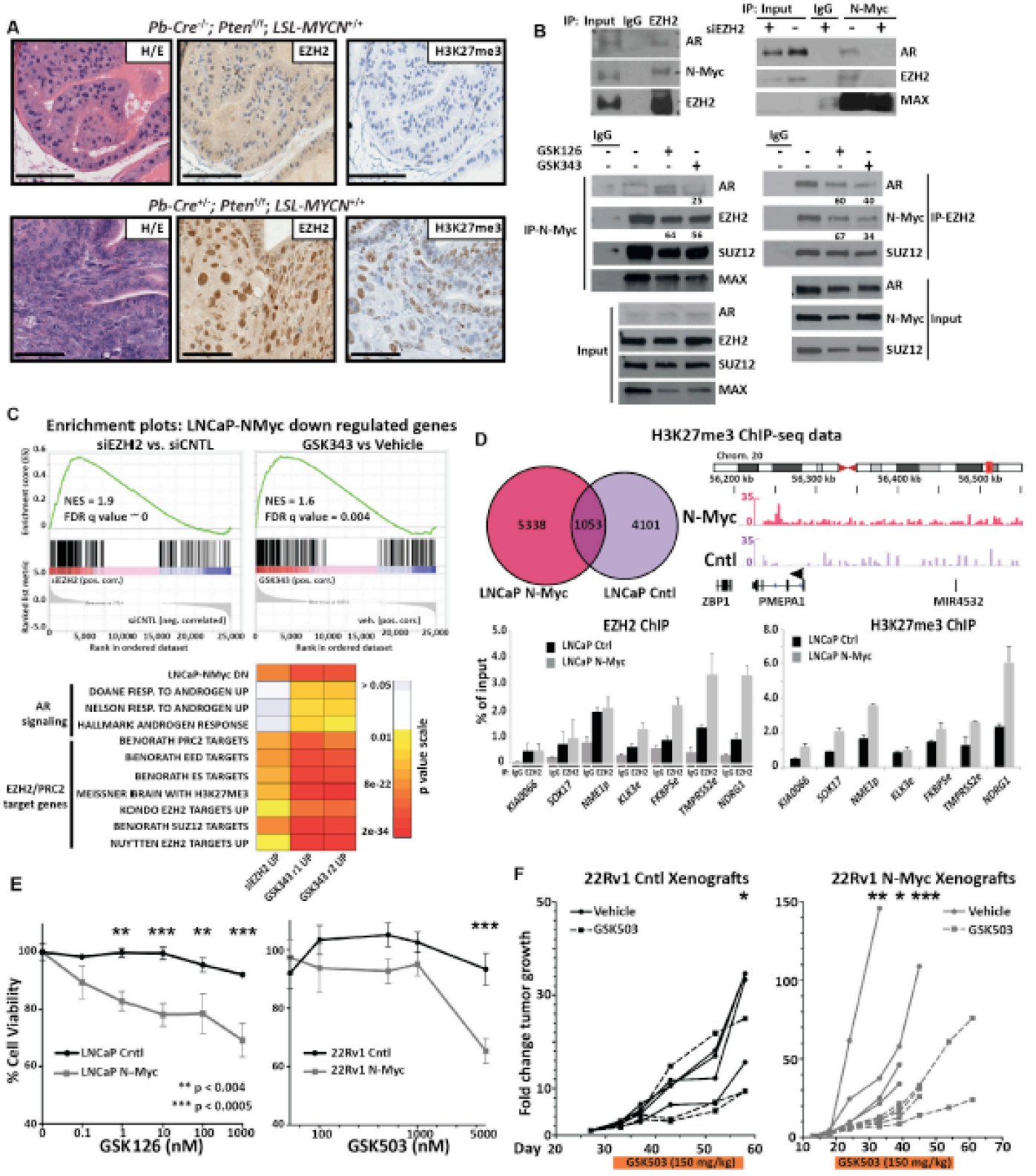


Fig 6. **A.** Photomicrographs (40x) of representative mouse prostates from indicated genotype following H/E staining, EZH2, and H3K27 tri-methylation (H3K27me3) IHC staining. **B.** Co-immunoprecipitation of N-Myc, EZH2, SUZ12 and AR upon EZH2 or N-Myc pull down in LNCaP-N-Myc cells and in LNCaP-N-Myc cells following knock-down of EZH2 with siRNA targeting EZH2 mRNA (top center), transfection of the Myc-tagged SET domain-deletion EZH2 mutant (top right) or 6-day treatment of either the EZH2 inhibitors GSK126 or GSK343 (bottom). Values below indicate the percent of interaction compared to vehicle if below70% **C.** Top: GSEA enrichment plot of the N-Myc down-regulated geneset in genes ranked in terms of comparison of LNCaP-N-Myc cells treated with siRNA targeting EZH2 versus control siRNA or GSK343 versus vehicle treatment; Bottom: heatmap of GSEA FDR q-values as shown in (**Fig. 3**) of AR induced genes and multiple PRC2 target gene sets that are significantly enriched in the N-Myc down-regulated genes that were significantly up-regulated after either siRNA-mediated EZH2 knock-down (48h) or treatment with GSK343 (7days, 5μM) in replicate. **D.** Top left: Venn diagram showing the overlap between H3K27me3 ChIP-seq reads enriched at promoters either in LNCaP-N-Myc or control (Cntl) cells; top right: H3K27me3 ChIP-seq reads in the indicated cells that were

localized at the chromosome loci housing the AR-regulated gene *PMEPA1*; bottom: EZH2 ChIP-PCR at known EZH2 binding sites for the indicated EZH2 target gene or negative control gene (*KIA0066*). **E.** Left: Percent cell viability of either LNCaP control (Cntl) or LNCaP-N-Myc (N-Myc) cells following 6 days incubation of the indicated dose of the EZH2 inhibitor GSK126. **F.** The fold change in growth rate of individual 22Rv1 control (Cntl, left) or N-Myc (right) xenografts before, during (orange bar below) and after 31 or 35 days (respectively) treatment with 150 mg/kg of the EZH2 inhibitor GSK503 (dashed lines) or vehicle (solid lines). Each tumor size at each time point was normalized to values obtained at day 1 of treatment.

Aim 2 Progress: The diagnosis of NEPC remains challenging and currently relies on a combination of pathologic and clinical features suggestive of AR signaling independence. There are no reliable blood biomarkers to consistently diagnose patients transforming to the NEPC phenotype. Detection of NEPC has clinical implications, as NEPC patients would not be expected to respond well to currently approved AR-targeted therapies for CRPC and may be better served by therapies specifically directed to NEPC. As part of this Aim, I have been using liquid biopsies to identify NEPC patients. I published initial work using circulating tumor cells (CTCs) during this grant period (Beltran et al, *Clinical Cancer Research* 2016). I am currently investigating ctDNA approaches as well using the molecular classifier developed in Aim 1 (in progress).

CTCs in NEPC: I recently characterized CTCs from patients with CRPC and NEPC utilizing the Epic Sciences platform (Epic Sciences, Inc, La Jolla, CA) and correlated results with patient-matched tumor biopsies and clinical features. Under an IRB approved protocol, patients with metastatic CRPC including those with pure or mixed NEPC were prospectively enrolled. NEPC was defined by the presence of either a pure or mixed small cell high-grade neuroendocrine carcinoma histology in a metastatic tumor biopsy and confirmed by at least 20% positive immunohistochemical staining for a neuroendocrine marker (synaptophysin, chromogranin). CRPC was defined clinically, with or without a metastatic biopsy confirming prostate adenocarcinoma. CRPC patients were sub-classified as atypical CRPC if the biopsy showed adenocarcinoma and the patient had clinical features suggestive of an AR independent transition which included radiographic progression in the setting of a low PSA <1 ng/ml, visceral progression in the absence of PSA progression (defined by Prostate Cancer Working Group 2 criteria and/or elevated serum chromogranin A >3X upper limit of normal.

CTCs from 27 patients with metastatic prostate cancer were evaluated. Overall, bone metastases were present in 24/27 (88.9%) of patients, and liver metastases were present in 8/12 (66.7%) of NEPC and 5/15 (33.3%) of CRPC of whom 4 had atypical clinical features. Median serum PSA level was 1.9 ng/ml in NEPC, 2.8 ng/ml in atypical CRPC, and 53.4 ng/ml in other CRPC patients. Serum neuroendocrine marker levels varied considerably within the NEPC subgroup and were also elevated in cases of CRPC. Two slides from each patient were evaluated by immunofluorescence (IF) using antibodies targeting cytokeratins (CK), CD45, AR, and 4',6-diamidino-2-phenylindole (DAPI) counterstain. Slides were imaged using a platform that captures all 3 million cells per slide in less than 15 minutes, and analyzed by a proprietary software that characterizes each cell by parameters including cell size, shape, nuclear area, presence of macronucleoli, CK and AR expression, uniformity and cellular localization. CK+/CD45- cells with intact, DAPI+ nuclei exhibiting tumor-associated morphologies were classified as traditional CTCs. CTCs with non-traditional characteristics were recorded, such as CK- /CD45- cells with morphological distinction and/or AR positivity, CK+/CD45- small cells, CTC clusters, CTCs with multiple marconucleoli and apoptotic CTCs (with nuclear or cytoplasmic fragmentation).

Enumeration of CTCs using both the CellSearch and Epic platforms was performed**.** Of note, 6/13 evaluated NEPC and atypical CRPC patients had CellSearch® CTC count of <5 CTC/7.5 mL (range 0-384, with 5 of these 13 patients having a CellSearch® CTC count of 0). In contrast, all 17 NEPC and atypical CRPC patients had CTCs ≥5 CTC/7.5mL using the Epic platform. Further characterization of the detected CTCs revealed heterogeneity of cytokeratin (CK) and AR expression in both NEPC and CRPC, with a significantly greater proportion of CK-negative and AR-negative CTCs in NEPC compared to CRPC**.** CTCs in NEPC patients overall had lower AR expression, higher cytoplasmic circularity, and higher nuclear to cytoplasmic ratio. The prevalence of CK-negative CTC subpopulations in NEPC patients is potentially consistent with a proposed epithelial-mesenchymal-transition (EMT). Based on the observed differences in CTCs between groups, we sought to identify CTC characteristics specific to NEPC. KDE analysis of the patient groups' CTCs in aggregate revealed significant differences in CK, AR and morphological characteristics when compared to CRPC. As a validation cohort, we evaluated baseline CTCs from 159 CRPC patients prospectively enrolled in an independent patient cohort at MSKCC for the presence of NEPC+ CTCs**.** NEPC+ CTC subpopulations

were identified in 17 of 159 (10.7%) cases. A significantly higher proportion of CRPC patients with visceral metastases harbored NEPC+ CTCs compared to those that were NEPC- (35% versus 15%, respectively; p=0.04). Patients with NEPC+ CTCs also had an overall higher CTC burden (median CTC count 64.6 versus 4.2; p<0.01)**.** In this proof of principle CTC Aim, I demonstrated that CTCs from patients with NEPC have distinct characteristics and thus their detection may potentially help identify patients that are developing NEPC-associated resistance. The results presented here indicate the feasibility of analyzing CTCs using the Epic platform and support the development of further studies to validate the clinical utility of CTCs for the early detection of patients transforming towards NEPC and the prognostic and potential predictive impact of CTC characteristics in predicting response to AR-directed therapies in CRPC.

Circulating tumor DNA (ctDNA) analysis in NEPC. In the first years of this Award, I have focused on specimen acquisition, protocol development, feasibility and reproducibility studies using ctDNA for CRPC and NEPC. This work has been in collaboration with F Demichelis, PhD (University of Trento), a computational biologist with expertise in prostate cancer genomics. During this grant year (2016), we completed whole exome sequencing (WES) of matched tumor biopsies, germline DNA, and ctDNA for 64 patients with metastatic CRPC/NEPC. After applying the partial duplication filtering method, we performed analysis for somatic copy number alterations (SCNA) and SNVs across the cohort. SCNA analysis was performed at different duplication levels. We used FACETS, a segmentation tool to that combines read count with informative SNP information (Shen et al, NAR 2016) and CLONET on all samples (plasma and tissues). SNV analysis was pefrormed using MuTECT and ASEQ on deduplicated samples. Overall the spectrum of genomic alterations captured in WES of ctDNA at <50 ng input DNA was consistent with those commonly observed in CRPC **(Figure 7)**, validating the feasibility of this approach to detect genome wide lesions non-invasively in patients using ctDNA.

Figure 7: Overview of copy number state of selected genes across study samples divided by levels of plasma tumor fraction.

Overall the genomic similarity of WES between matched ctDNA and NEPC tumor biopsies was higher than between ctDNA and CRPC –Adeno (Fig 8), suggesting potentially less heterogeneity in the late stage NEPC phenotyp . The relative contribution of ctDNA based on size and site of metastases (liver vs. bone, for instance) is currently being explored.

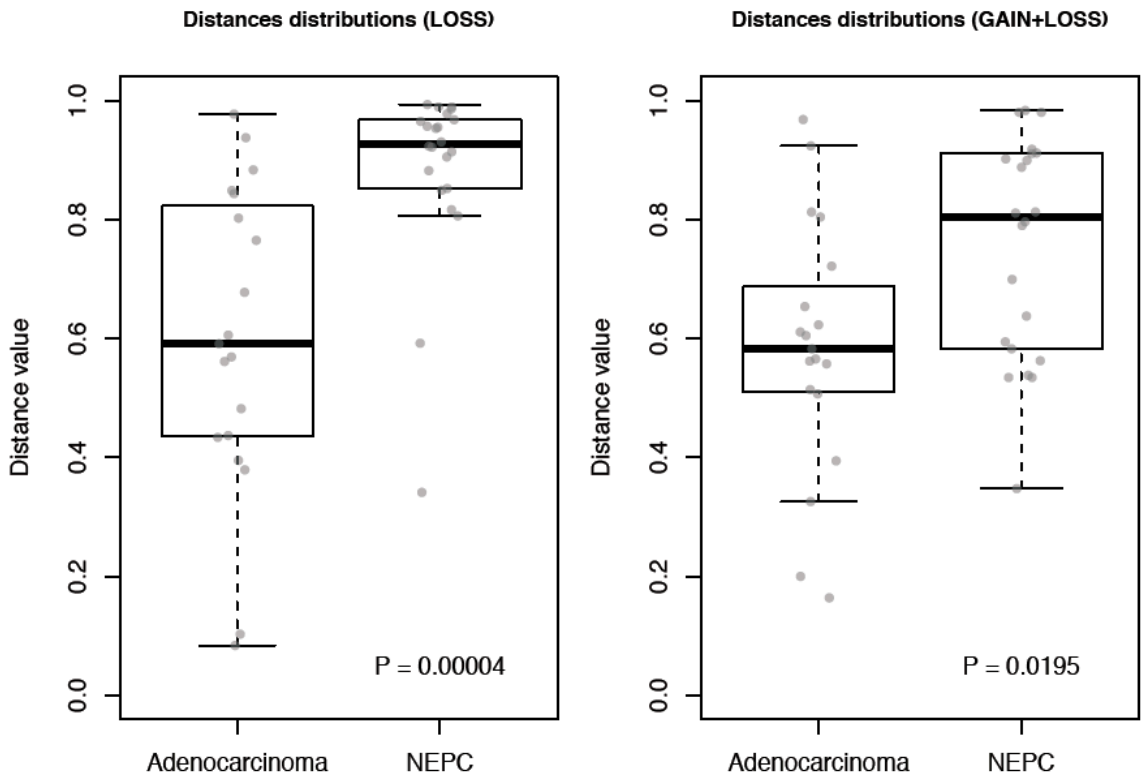


Figure 8: Intra-patient similarities between WES data from metastatic biopsies (CRPC-Adeno or NEPC) and ctDNA.

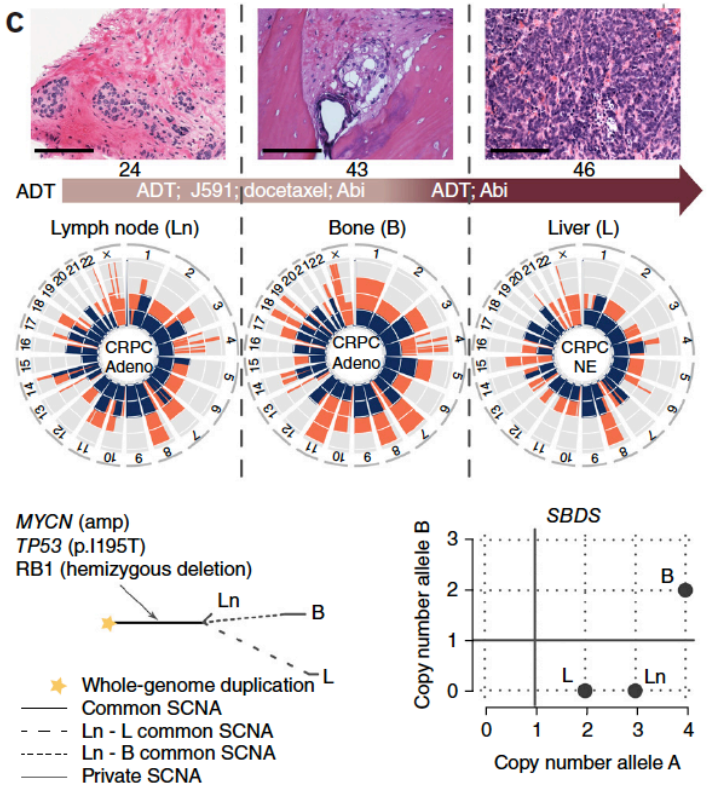


Figure 9: Allele-specific analysis of tumors at three time points from PM161: lymph node (CRPC-Adeno), bone biopsy (CRPC-Adeno) and liver biopsy (NEPC). Middle, Circos plots of genome-wide allele-specific DNA quantity. Bottom left, phylogenetic tree built from allele specific copy number data. Bottom right, allele-specific copy number of *SBDS*.

We evaluated serial tumor biopsies from an individual patient (PM161), a patient who developed progression of disease after multiple lines of therapy for CRPC—including the development of new visceral metastases at the time of progression on abiraterone with a liver biopsy at progression showing small-cell carcinoma **(Figure 12).** We compared three different time points—CRPC-Adeno (adenocarcinoma, lymph node metastasis), CRPC-Adeno (adenocarcinoma, bone metastasis) and NEPC (small-cell carcinoma, liver metastasis at progression on abiraterone therapy). Whole exome sequencing analysis of the tumors suggested divergent clonal evolution (Beltran et al, Nat Med 2016). In other words, NEPC appeared clonal in origin with a clonal ancestry traceable back to a CRPC-Adeno precursor.

To extend upon these findings, we evaluated patient PM161's ctDNA by WES at baseline and compared the data to all three tissue time-points **(Figure 10).** Unexpectedly the baseline ctDNA profile (at time of CRPC-Adenocarcinoma diagnosis) displayed genomic features most similar to the NEPC tissue sample (last time-point, liver biopsy). These data suggest that NEPC alterations are detectable and the circulation and they may potentially be detected prior to the clinical development of NEPC clinical and histologic features. These results have important potential clinical implications for early detection. We are currently extending ctDNA to a larger number of patients.

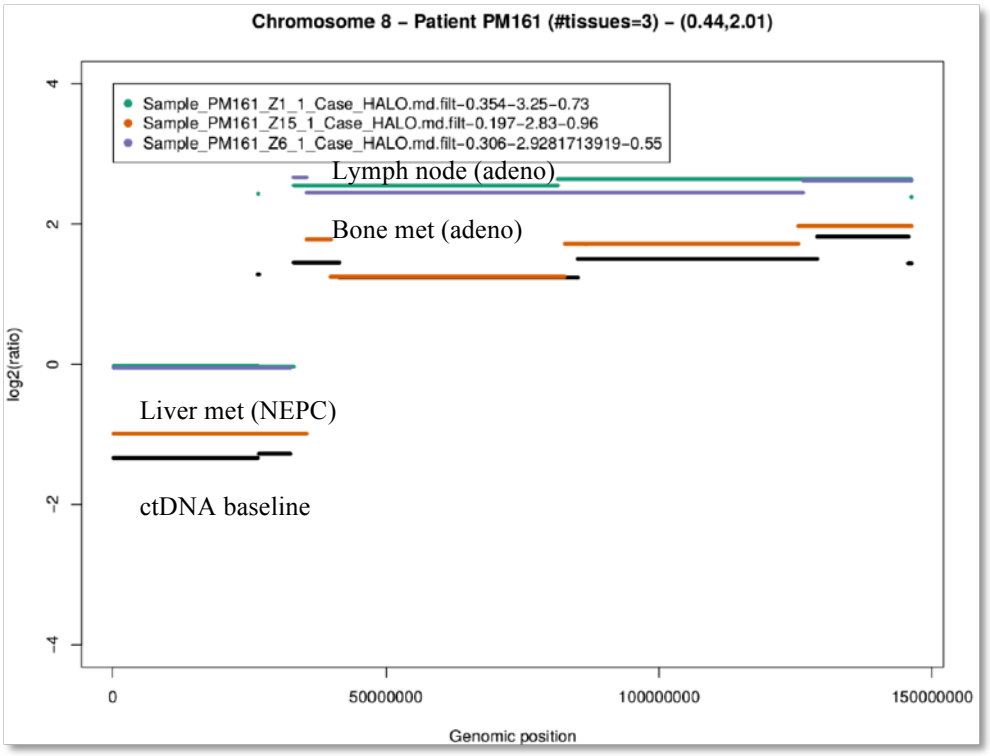


Figure 10: Somatic copy number (SCNA) and CLONET comparison between tumoral tissue samples of patient PM161 at the 3 metastatic biopsy time-points compared with plasma SCNA profile at baseline (same time-point as LN) at representative area of genome (chromosome 8 shown).

Aim 3 Progress: I have been systematically evaluating high-risk primary prostate tumors for mutations that may predispose to resistance to AR targeted therapy including the development of NEPC (ongoing work). For instance, we compared multiple primaries (high grade adenocarcinoma) and multiple metastases in an individual patient who died from NEPC and had a rapid autopsy case. We reconstructed a phylogenetic tree of all samples, shown in **Figure 11** and elucidated the clonal evolution. Further, we also evaluated ctDNA from plasma sample obtained before death and performed WES and compared to each metastatic site. On average approximately 50% of mutations found in tumor tissue was present and detectable in ctDNA and higher for liver vs. lymph node. This was performed in order to gain insights into the contribution of each site in the circulation.

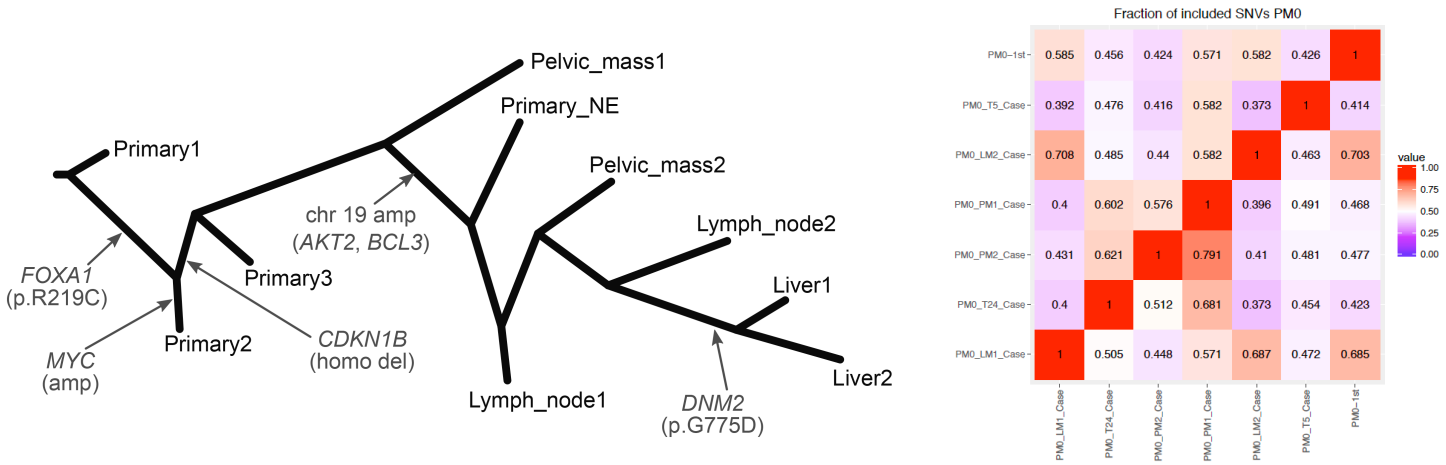


Figure 11: Left: phylogenetic tree depicting SCNA and tumor evolution in PM0 with comparison of primary tumor (n=3) and multiple metastases at time of rapid autopsy (n=7). Right= Degrees of similarity of WES SCNA data between multiple metastatic tissue samples at autopsy compared with plasma SCNA profile.

Also as part of this Aim and in collaboration with Dr. Gleave (Vancouver), Dr. Wyatt (Vancouver), and Dr. Halabi (Duke), I have been evaluating localized prostate cancer samples collected through the Phase 3 CALGB

90203 Trial*, "Immediate prostatectomy vs. neoadjuvant docetaxel and androgen deprivation therapy for men with high risk, localized prostate cancer"* to assess the impact of therapy on modulation of gene expression of a panel of neuroendocrine prostate cancer (NEPC) pathway signature genes. This includes a 169 gene panel developed and validated by my lab, including AR regulated genes, neural and neuroendocrine marker genes, epithelial mesenchymal genes, cell cycle genes, and others credentialed to distinguish NEPC from prostate adenocarcinoma. This data will be correlated with clinical and pathologic characteristics including AR and neuroendocrine marker immunohistochemistry (IHC), other correlative studies as part of this trial (including ETS fusions status and genomic mutation and copy number profiles), and clinical outcomes (data expected to read out late 2017). Strengths of this project include utilization of a novel NEPC signature Nanostring assay that can be performed using limited material from formalin fixed paraffin embedded (FFPE) tissues. RNA expression analyses of neoadjuvant-treated prostatectomy specimens have been challenged in the past due to microscopic residual foci and the necessity for fresh/frozen material. Our NEPC signature Nanostring assay demonstrates significant discrimination between NEPC and adenocarcinoma and has shown high correlation with RNA-seq data(Spearman coefficient 0.9), and therefore represents a significant strength to this study.

During this grant period in 2016, we evaluated 45 untreated and post-treatment FFPE specimens as well as patient-matched pre-treated needle biopsies and baseline clinical data and these initial results were presented at GU ASCO this year (Beltran et al, *GU ASCO 2016, manuscript in preparation*). Molecular subsets emerged on unsupervised analysis (Fig 12). There was significant upregulation of AR and the ARv7 expression following treatment, as well as a subset of NEPC and EMT genes; three high chromogranin A outlier cases were identified in the treatment arm. There was an overall higher AR score in treated cases (based on expression of 30 AR signaling genes) compared to untreated, along the spectrum of CRPC. These data support the feasibility of quantifying gene expression in neoadjuvant-treated high risk localized PCA cases with limited FFPE tissue requirement. Extensive characterization of AR status and NE/EMT genes identifies molecular outliers that can arise post-treatment and provides new insight into the heterogeneity of treatment response and potential early markers of resistance. We have extended this analysis to 200 samples, integrated RNA data with DNA, and expect to report these results in 2017. The detection and determination of frequency of early NEPC-associated alterations may have significant prognostic and treatment implications in helping identify high-risk, clinically localized prostate cancers as harbingers of resistant disease.

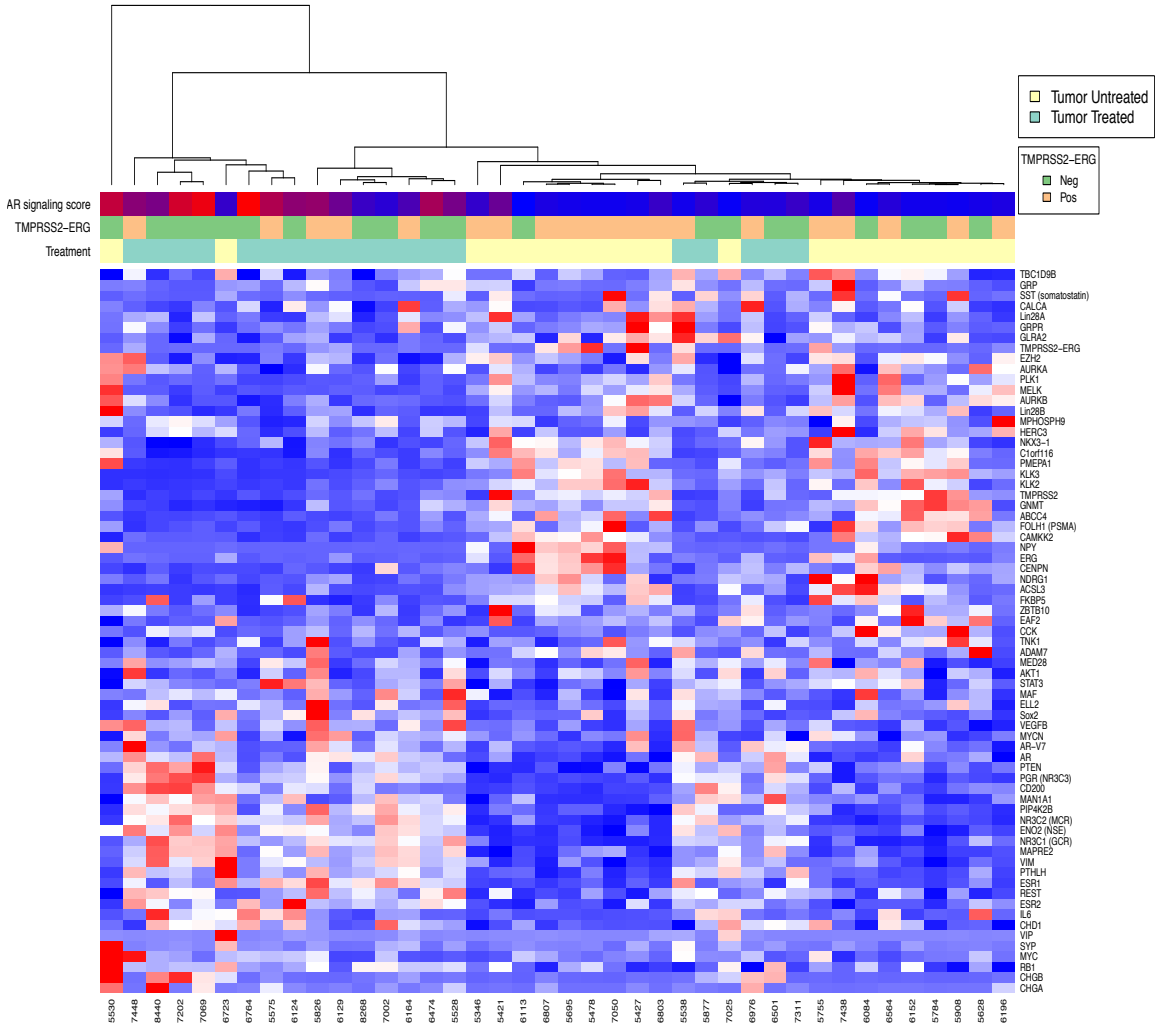


Fig 12: Unsupervised analysis of differentially expressed genes in prostatectomy specimens from treated and untreated groups in the Phase 3 CALGB 90203 Trial including AR signalling and NEPC genes. Red= high expression, Blue= low expression.

KEY ACCOMPLISHMENTS:

- Extensive molecular analysis including whole exome, methylome, and transcriptome sequencing of CRPC-Adeno and CRPC-NE metastatic tumors (and matched primaries) with clinical correlation (Beltran et al, *Nature Medicine*, 2016)
- Collection and molecular characterization of CTCs from CRPC-Adeno and CRPC-NE patients (Beltran et al, *CCR*, 2016)
- Establishment the largest tissue Biorepository of neuroendocrine prostate cancer
- First in field circulating tumor DNA analysis for NEPC including whole exome sequencing ctDNA
- Establishment of a Precision Medicine Clinic and Rapid Autopsy program at Weill-Cornell-NYP for enrolling advanced cancer patients under an IRB approved protocol to understand mechanism of resistance during disease progression and at the time of death (five prostate cancer rapid autopsies performed to date).
- Correlative analysis of neoadjuvant treated specimens in the Phase 3 trial CALGB90203 (Beltran et al, GU ASCO 2016) and metastatic samples from the Phase 2 alisertib trial for NEPC (Beltran et al ESMO 2016).

5. **CONCLUSION:** This Award has allowed me to evaluate mechanisms of prostate cancer resistance to AR targeted therapies by performing integrative genomic and epigenomic analyses of metastatic tumors from patients with castration resistant prostate cancer. I have focused on the development of an AR indifferent neuroendocrine phenotype, as this has recently emerged as an aggressive phenotype that is challenging to diagnose and treat. I am using this knowledge to develop biomarkers to improve diagnosis and early detection

of patients developing NEPC. I have evaluated CTCs and more recently cell-free DNA in plasma of treated patients at different time points for the emergence of subsets of cells with resistance-associated alterations, as this may serve as a noninvasive method to detect altered genes in an individual patient. I am also looking at high risk localized prostate tumors with and without neoadjuvant therapy for the presence or emergence of NEPC features. With continued work, this project has high potential for further validation and clinical development of biomarkers and could directly influence patient care by identifying patients less likely to respond to subsequent AR –directed therapy and who could be selected for alternative NEPC directed therapeutic approaches. This data has also identified novel drivers of treatment resistance and has nominated therapeutic targets for further preclinical development. This Phyisican Training Award has greatly facilitated my career development, directly resulting in several academic collaborations, grants, and manuscripts

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

(1) Lay Press:

Himisha Beltran, Synthetic Lethality and Beyond, *Science Translational Medicine,* 16 Nov 2016: Vol. 8, Issue 365, pp. 365ec182 DOI: 10.1126/scitranslmed.aal0070

Himisha Beltran, How to Make a Cocktail, *Science Translational Medicine,* 05 Oct 2016: Vol. 8, Issue 359, pp. 359ec158 DOI: 10.1126/scitranslmed.aai8745.

Himisha Beltran, Networking and Matchmaking in Prostate Cancer, *Science Translational Medicine,* 24 August 2016: Vol. 8, Issue 353, pp. 353ec133.

Himisha Beltran, EZH2 drives and defines an epigenetic subclass of lung cancer, *Science Translational Medicine,* 13 July 2016: Vol. 8, Issue 347, pp. 347ec110.

Himisha Beltran. Confronting the Challenges of Precision Oncology, *Science Translational Medicine,* 11 June 2016: Vol. 8, Issue 341, pp. 341ec86**.**

Himisha Beltran. Cancer Metastases: Are one and all the same? *Science Translational Medicine,* 06 April 2016: Vol. 8, Issue 333, pp. 333ec57

(2) Peer-Reviewed Scientific Journals during Year 3:

1. Panagiotis Vlachostergios, Loredana Puca, **Himisha Beltran***, Emerging Variants of Castration-resistant Prostate Cancer, *Current Oncology Reports, in press.* *Corresponding author*

2. Ping Mu, Zeda Zhang, Matteo Benelli, Wouter Karthaus, Elizabeth Hoover, John Wongvipat, Sheng-Yu Ku, Chi-Chao Chen, Dong Gao, Zhen Cao, Neel Shah, Elizabeth Adams, Wassim Abida, Philip A. Watson, Chun-Hao Huang, Elisa De Stanchina, Scott Lowe, Leigh Ellis, **Himisha Beltran**, Mark A. Rubin, David Goodrich, Francesca Demichelis, Charles L. Sawyers, *SOX2* promotes lineage plasticity and antiandrogen resistance in *TP53* mutant prostate cancer, *Science, in press.*

3. Cora Sternberg and **Himisha Beltran**, **Improved outcomes and precision medicine come within reach**, *Nature Reviews Urology, in press*.

4. Etienne Dardenne[#], **Himisha Beltran[#] (co-first author),** Matteo Benelli, Kaitlyn Gayvert, Adeline Berger, Loredana Puca, Joanna Cyrta, Andrea Sboner, Zohal Noorzad, Theresa MacDonald, Cynthia Cheung, Dong Gao, Yu Chen, Martin Eilers, Juan-Miguel Mosquera, Brian D. Robinson, Olivier Elemento, Mark A. Rubin, Francesca Demichelis, David S. Rickman, N-Myc drives Aggressive Prostate Cancer and the Neuroendocrine Phenotype, *Cancer Cell, in press.* Published online ahead of print 10 October 2016.

5. Bishoy Faltas, Davide Prandi, Scott T. Tagawa, Ana Molina, David M.Nanus, Cora Sternberg, Jonathan

Rosenberg, Juan Miguel Mosquera, Brian Robinson, Olivier Elemento, Andrea Sboner, **Himisha Beltran* (co-senior author),** Francesca Demichelis*, Mark A. Rubin*, Clonal Evolution of Chemotherapy-Resistant Urothelial Carcinoma, *Nature Genetics, in press.* Published online ahead of print 17 October 2016.

6. Bishop JL, Thaper D, Vahid S, Jama R, Ketola K, Kim S, Davies A, Angeles A, Sangha B, Kuruma H, Nip KM, Wyatt A, Gleave ME, Wang YZ, Collins C, **Beltran H**, Zoubeidi A, The master neural transcription factor BRN2 is an androgen receptor suppressed driver of neuroendocrine differentiation in prostate cancer, *Cancer Discovery, in press.* Published online ahead of print 26 October 2016.

7. Rennert H, Eng K, Zhang T, Tan A, Xiang J, Romanel A, Kim R, Tam W, Liu YC, Bhinder B, Cytra J, **Beltran H**, Robinson B, Mosquera JM, Fernandes H, Demichelis F, Sboner A, Kluk M, Rubin MA, Elemento O. Development and Validation of a Whole Exome Sequencing Test-1 (EXaCT-1) for Simultaneous Detection of Point Mutation, Indels and Copy Number Alterations for Precision Cancer Care, *npj Genomic Medicine,* 2016 July, 16019, doi:10.1038/npjgenmed.2016.19.

8. **Beltran H**, Antonarakis ES, Morris MJ, Attard G, Emerging Molecular Biomarkers in Advanced Prostate Cancer: Translation to the Clinic, *ASCO Education Book*. 2016; 35:131-41. **Corresponding author*

9. Colin C. Pritchard, Joaquin Mateo, Michael Walsh, Navonil De Sarkar, Wassim Abida, **Himisha Beltran**, Roman Gulati, Suzanne Carreira, Rosalind Eeles, Olivier Elemento, Mark A. Rubin, Dan Robinson, Robert Lonigro, Arul Chinnaiyan, Jake Vinson, Julie Filipenko, Andrea Garofalo, Levi Garraway, Mary Ellen Taplin, Saud Al Dubayan, Garam C Han, Mallory Beightol, Colm Morrissey, Jennifer Noteboom, Belinda Nghiem, Heather H. Cheng, Bruce Montgomery, Tom Walsh, Silvia Casadei, Vijai Joseph, Howard Scher, Charles Sawyers, Nikolaus Schultz, Phil Kantoff, David Solit, Mark Robson, Eli Van Allen, Kenneth Offit, Johann De Bono, Peter S. Nelson, Inherited DNA Repair Gene Mutations in Men with Metastatic Prostate Cancer, *New England Journal of Medicine,* 2016 Aug 4;375(5):443-53. doi: 10.1056/NEJMoa1603144.

10. Li Y, Donmez N, Xie N, Wang Y, Xue H, **Beltran H,** Sahinalp C, Gleave M, Wang Y, Colin Collins C, Dong X. SRRM4 Induces Neuroendocrine Transdifferentiation of Prostate Cancer Cells under AR Pathway Inhibition, *European Urology, in press.* 2016 May 11. pii: S0302-2838(16)30138-5 [Epub ahead of print]

11. **Himisha Beltran***, Davide Prandi, Juan Miguel Mosquera, Matteo Benelli, Loredana Puca, Joanna Cyrta, Clarisse Marotz, Eugenia Giannopoulou, Balabhadrapatruni V.S.K. Chakravarthi, Sooryanarayana Varamball, Scott Tomlins, David M. Nanus, Scott T. Tagawa, Eliezer M. Van Allen, Olivier Elemento, Andrea Sboner, Levi Garraway, Mark A. Rubin*, Francesca Demichelis*, Divergent clonal evolution of castration resistant neuroendocrine prostate cancer, *Nature Medicine*, Mar;22(3):298-305. doi: 10.1038/nm.4045. **Corresponding author*

12. **Himisha Beltran***, Adam Jendrisak, Mark Landers, Juan Miguel Mosquera, Myriam Kossai, Jessica Louw, Rachel Krupa, Ryon Graf, David M Nanus, Scott T Tagawa, Dena Marrinucci, Ryan Dittamore, Howard Scher, Initial detection and partial characterization of circulating tumor cells in neuroendocrine prostate cancer, *Clinical Cancer Research,* 2016 Mar 15;22(6):1510-9. **Corresponding author*

13. S. Gillessen, A. Omlin, G. Attard, J. S. de Bono, E. Efstathiou, K Fizazi, S. Halabi, P. S. Nelson, O. Sartor, M. R. Smith, H. R. Soule, H., Akaza, T. M. Beer, **H. Beltran**, A. M. Chinnaiyan, G. Daugaard, I. D. Davis, M. De Santis, C. G. Drake, R. A. Eeles, S. Fanti, M. E. Gleave, A. Heidenreich, M. Hussain, N. D. James, F. E. Lecouvet, C. J. Logothetis, K, Mastris, S. Nilsson, W. K. Oh, D. Olmos, A. R. Padhani, C. Parker, M. A. Rubin, J. A. Schalken, H. I. Scher, A. Sella, N. D. Shore, E. J. Small, C. N. Sternberg, H. Suzuki, C. J. Sweeney, I. F. Tannock, B. Tombal, Management of patients with advanced prostate cancer: highlights of the St Gallen Advanced Prostate Cancer Consensus Conference (APCCC), *Annals of Oncology*, 2016 May 2. pii: mdw180.

14. Pauli C, Puca L, Mosquera JM, **Beltran H**, Rubin MA, Rao RA. An Emerging Role For Cytopathology In Precision Oncology, *Cancer Cytopathology,* 2016 Mar;124(3):167-73. doi: 10.1002/cncy.21647.

(3) Invited Articles:

Himisha Beltran, Update on the biology and management of neuroendocrine prostate cancer, *Clin Adv Hematol Oncol*. 2016 Jul;14(7):513-5.

(4) Abstracts: List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.

Beltran H, Danila D, Montgomery B, Szmulewitz R, Vaishampayan U, Armstrong A, Hoimes C, Stein M, Pinski J, Scher H, Puca L, Bareja R, Wong W, Rubin MA, Mosquera JM, Sboner A, Oromendia C, Nanus DM, Ballman K, Tagawa ST, A phase 2 study of the aurora kinase A inhibitor alisertib for patients with neuroendocrine prostate cancer (NEPC), **Oral Abstract Presentation**, ESMO Annual Meeting, 2016.

Beltran H, Prandi D, Mosquera JM, Giannopoulou E, Puca L, Marotz C, Nanus DM, Tagawa ST, Elemento O, Van Allen E, Sboner A, Garraway L, Rubin MA, Demichelis F. *Divergent Clonal Evolution of Castration Resistant Neuroendocrine Prostate Cancer*, **Oral Abstract Presentation**, Department of Defense Prostate Cancer Research Program (PCRP) Innovative Minds in Prostate Cancer Today (IMPaCT) Conference, Baltimore, MD, 2016.

Loredana Puca, Dong Gao, Myriam Kossai, Clarisse Marotz, Andrea Sboner, Juan Miguel Mosquera, Theresa Y. MacDonald, Kyung Park, Rema Rao, Andrea Sboner, Yu Chen, Mark A. Rubin, **Himisha Beltran**. *Targeting EZH2 in the AR-independent advanced prostate cancer with neuroendocrine features*, **Poster Presentation**, AACR 2016, New Orleans 2016.

Etienne Dardenne[#], **Himisha Beltran[#],** Matteo Benelli, Kaitlyn Gayvert, Adeline Berger, Loredana Puca, Joanna Cyrta, Andrea Sboner, Zohal Noorzad, Theresa MacDonald, Cynthia Cheung, Dong Gao, Yu Chen, Martin Eilers, Juan-Miguel Mosquera, Brian D. Robinson, Olivier Elemento, Mark A. Rubin, Francesca Demichelis, David S. Rickman, N-Myc drives Aggressive Prostate Cancer and the Neuroendocrine Phenotype, **Minisymposium Presentation**, AACR 2016, New Orleans 2016.

Beltran H, Wyatt A, Chedgy E, Fazli L, Sboner A, Halabi S, Gleave ME, *Impact of therapy on gene expression in high-risk prostate cancer treated with neoadjuvant docetaxel and androgen deprivation therapy*, **Poster presentation**, GU ASCO 2016, San Francisco, CA.

7. **INVENTIONS, PATENTS AND LICENSES:** Nothing to report

8. **REPORTABLE OUTCOMES:** Nothing to report

9. **OTHER ACHIEVEMENTS in YEAR 3:**

- Prostate Cancer Foundation Challenge Award

10. **REFERENCES:**

Ping Mu, Zeda Zhang, Matteo Benelli, Wouter Karthaus, Elizabeth Hoover, John Wongvipat, Sheng-Yu Ku, Chi-Chao Chen, Dong Gao, Zhen Cao, Neel Shah, Elizabeth Adams, Wassim Abida, Philip A. Watson, Chun-Hao Huang, Elisa De Stanchina, Scott Lowe, Leigh Ellis, **Himisha Beltran**, Mark A. Rubin, David Goodrich, Francesca Demichelis, Charles L. Sawyers, *SOX2* promotes lineage plasticity and antiandrogen resistance in *TP53* mutant prostate cancer, *Science, in press.*

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Phenotype, *Cancer Cell, in press.* Published online ahead of print 10 October 2016.

Bishop JL, Thaper D, Vahid S, Jama R, Ketola K, Kim S, Davies A, Angeles A, Sangha B, Kuruma H, Nip KM, Wyatt A, Gleave ME, Wang YZ, Collins C, **Beltran H**, Zoubeidi A, The master neural transcription factor BRN2 is an androgen receptor suppressed driver of neuroendocrine differentiation in prostate cancer*, Cancer Discovery, in press.* Published online ahead of print 26 October 2016.

Himisha Beltran*, Davide Prandi, Juan Miguel Mosquera, Matteo Benelli, Loredana Puca, Joanna Cyrta, Clarisse Marotz, Eugenia Giannopoulou, Balabhadrapatruni V.S.K. Chakravarthi, Sooryanarayana Varamball, Scott Tomlins, David M. Nanus, Scott T. Tagawa, Eliezer M. Van Allen, Olivier Elemento, Andrea Sboner, Levi Garraway, Mark A. Rubin*, Francesca Demichelis*, Divergent clonal evolution of castration resistant neuroendocrine prostate cancer, *Nature Medicine*, Mar;22(3):298-305. doi: 10.1038/nm.4045. **Corresponding author*

Himisha Beltran, Kenneth Eng, Juan Miguel Mosquera, Alexandros Sigaras, Alessandro Romanel, Hanna Rennert, Myriam Kossai, Chantal Pauli, Bishoy Faltas, Jacqueline Fontugne, Kyung Park, Jason Banfelder, Davide Prandi, Neel Madhukar, Tuo Zhang, Jessica Padilla, Noah Greco, Terra J. McNary, Erick Herrscher, David Wilkes, Theresa Y. MacDonald, Hui Xue, Vladimir Vacic, Anne-Katrin Emde, Dayna Oschwald, Adrian Y. Tan, Colin Collins, Martin E. Gleave, Yuzhuo Wang, Dimple Chakravarty, Marc Schiffman, Rob Kim, Fabien Campagne, Brian D. Robinson, David M. Nanus, Scott T. Tagawa, Jenny Z. Xiang, Agata Smogorzewska, Francesca Demichelis, David Rickman, Andrea Sboner, Olivier Elemento, Mark A. Rubin*. A Precision Medicine Trial for Whole Exome Sequencing of Metastatic Cancer Reveals Biomarkers of Treatment Response*, JAMA Oncology,* 2015 Jul 1;1(4):466-74

Gao D ,Vela I, Sboner A, Iaquinta PJ, Karthaus WP, Gopalan A, Wajala JN, Dowling C, Undvall EA, Arora VK, Wongvipat J, Kossai M, Ramazanoglu S, Barboza LP, Di W, Cao Z, Zhang QF, Sirota I, Ran L, MacDonald TY, Beltran H, Mosquera JM, Touijer KA, Scardino PT, Laudone VP, Curtis K, Rathkopf DE, Morris MJ, Danila DC, Slovin SF, Solomon SB, James A. Eastham JA, Chi P, Carver B, Rubin MA, Scher HI, Clevers H, Sawyers CL,Chen Y. Generation of *in vitro* organoid cultures derived from patients with advanced prostate cancer. *Cell, 2015*

11. **APPENDICES:** N/A